

In Vitro Propagation Studies in *Gymnema* (*Gymnema Sylvestre* R. Br.)

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Abstract

Tissue culture technology is used for selection and rapid multiplication. In India the micro propagation technique has gained momentum to reoccupy the monopoly in *Gymnema* tissue culture at global level. It is able to regenerate millions of copies to ensure in a decade of time with high yielding with shorter duration. There is a tremendous demand for natural anti diabetic agents, because it have reducing blood sugar and are used as an anti diabetic agent in Indian medicine, improvement of cultivation is carried out by the in-vitro culturing or tissue culture technique. In-vitro studies or micro propagation of *Gymnema* were conducted at Agricultural College & Research institute, Killikulam at 2001. The shoot tip and nodal segments were collected and used as explants. These explants of *Gymnema* were inoculated in MS medium (Murashige and Shoog, 1962) supplemented with Kinetin (2.5 mg/lit), NAA (1 mg/lit), and GA3 (2.0 mg/lit) gave the better results for callus induction and shoot formation.

Keywords: *Gymnema*; Tissue culture; Kinetin; NAA.

Introduction

Gymnema sylvestris R.Br. belongs to the family Asclepiadaceae known as Sirukurujan and Gurmar. It is a woody climber and the leaves possess active principles like gymnemic acid, which are reported to have properties of reducing sugar and are used as an antidiabetic agent in Indian systems of medicine. So there is a tremendous demand for gymnema production. In India micro propagation technique has gained momentum to reoccupy the monopoly

in gymnema production at global level. It is able to regenerate million of genetically identical copies of high yielding types in a shorter time and space, and the tissue cultured plants from different explants perform uniformly.

Materials and Methods

The successful *in-vitro* culture results depend on the interplay of the plant material (explants),

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medium in use and the culture environment. Two different explants namely shoot tip and nodal segments are involved in this study. The explants were surface sterilized with 0.1 per cent mercuric chloride for 10 minutes followed by washed with distilled water. Then it is rinsed with 70% ethanol for 3 times then it is washed with distilled water. Now the explants were ready for inoculation. The medium contained sucrose and solidified with 0.8% agar, p^H of the media was adjusted to 5.7 before autoclaving at 121°C for 20 minutes. For callus growth and direct regeneration required quantity of auxins (NAA), cytokinin (BAP) and GA_3 were added in MS medium. Cultures were maintained at $27 \pm 2^\circ C$ temperature, 75% relative humidity and with 16 hours photoperiod.

Treatments involved

T₁ - Normal MS media + BAP (2.0 mg /lit) + NAA (1.0 mg/lit)

T₂ - Normal MS media + Kinetin (2.5 mg/lit) + NAA (1.0 mg/lit)

T₃ - Normal MS media +BAP (2.0 mg/lit) + NAA (1.0 mg/lit) + GA_3 (2.0 mg/lit)

T₄ - Normal MS media + Kinetin (2.5 mg/lit) + NAA (1.0 mg/lit) + GA_3 (2.0 mg/lit)

Results and Discussion

In the present investigation, growth regulator combination of kinetin (2.5 mg/lit) + NAA (1.0 mg/lit) and Kinetin (2.5 mg/lit), NAA (1.0 mg/lit) and GA_3 (2.0 mg/lit) gave the better result for callus induction and shoot proliferation. The use of kinetin in the culture medium has been reported by Anu *et al.* [1] and Somany *et al.* [3]. The shoot tip explant gave the maximum callus establishment (64.5%) and shoot regeneration in the T₄ treatment followed by T₂ treatment. (Table 1). The nodal segment also gave the better establishment of callus induction in T₂ treatment. The higher proportion of shoot proliferation from nodal segment followed by shoot tip was obtained on ms medium supplemented with Kinetin (2.5 mg/lit) and NAA (1.0 mg/lit) and GA_3 (2.0 mg/lit) (Table 2). These results are in agreement with the findings of Lakshmisita *et al.* [2].

Table 1: Response of explants of *Gymnema* in different treatments of MS media

Callus induction medium	Response of explants to callus induction and shoot proliferation	
	Shoot tip	Nodal segment
T ₁	C (57.2%)	-
T ₂	C (63.3) and S	C (57.5%)
T ₃	C (52.4%)	-
T ₄	C (64.5%) and S	C (48.7%)

- : No callus production C : Callus production

CS : Callusing and shoot proliferation

Table 2: Shoot regeneration responded by explants

Callus induction medium	Response of explants to shoot regeneration	
	Nodal segment	Shoot tip
T ₁	✓	*
T ₂	✓	*
T ₃	✓	*
T ₄	✓	**

✓-Shoot regeneration

*- Shoot proliferation

** - Proliferation is abundant

Summary

In the present investigation of *Gymnema* the shoot tip explant shows good callus growth and shoot proliferation in ms media supplemented with Kinetin (2.5 mg/lit) , NAA (1.0 mg/lit) and GA_3 (2.0 mg/lit), hence the above said treatment will be used for tissue culture in *Gymnema*.

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